analytical chemistry

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Fluoroalkylamines: Novel, Highly Volatile, Fast-Equilibrating, and Electrospray Ionization–Mass Spectrometry Signal-Enhancing Cationic Ion-Interaction Reagents

Bassam Lajin* and Walter Goessler

Cite This: *Anal. Chem.* 2020, 92, 10121–10128

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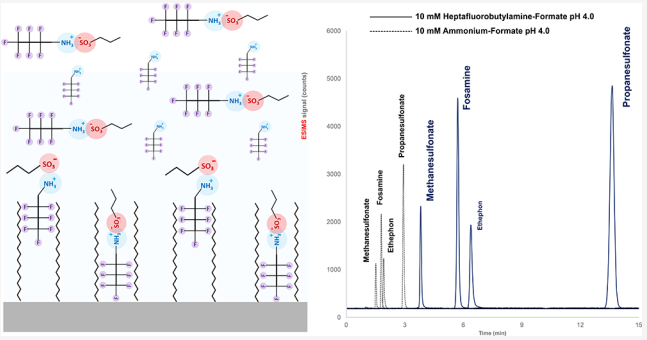
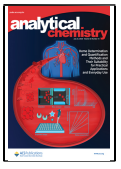
ABSTRACT: A new class of cationic ion-interaction reagents for reversed-phase chromatography is introduced in the present work. Compounds belonging to a homologous series of linear fluoroalkyl chains including trifluoroethylamine (TFEAm), pentafluoropropylamine (PFPAm), heptafluorobutylamine (HFBAm), and nonafluoropentylamine (NFPAm) were tested and compared with ammonia and triethylamine (TEA) for the separation of selected organic acids of general interest such as the herbicides glyphosate, ethephon, and fosamine and arsenic metabolites methylarsonic acid and dimethylarsinic acid as well as other compounds. Depending on the carbon and fluorine atom number, the fluoroalkylamines were shown to be effective cationic ion-interaction reagents, significantly enhancing the retention of organic acids on a C18 reversed-phase column. Contrary to the general behavior of ion-interaction reagents (a broader term than ion-pairing reagent), significant (up to 5-fold) and consistent enhancement in the electrospray ionization mass spectrometry signal (ESI-MS) was observed relative to ammonia and triethylamine. Overall, among the tested series HFBAm was found to offer the best overall properties among the tested series as it provided a good compromise between column equilibration time (ca. 25 column volumes) and retention behavior (up to a 10-fold increase in the retention factor of acids relative to ammonia) while providing the same general advantages found for the fluoroalkylamines such as fast washout times from the ESIMS system (ca. 30 min) and a 3–5-fold signal enhancement. The fluoroalkylamines are a new class of cationic ion-interaction reagents with clear advantages over the currently employed alkylamines and may revive the general interest in ion-interaction chromatography.



Ion-interaction chromatography (more commonly but less accurately called ion-pair chromatography) is used to improve the retention of strongly ionized compounds in order to achieve their separation simultaneously with neutral compounds while taking advantage of the most commonly used and versatile reversed-phase stationary phases and has been used since the 1970s.[1−4] Since the incorporation of the ion-interaction[5,6] mechanisms in reversed-phase chromatography, its application gradually subsided as it was observed to be associated with several disadvantages, including very long equilibration and washout times, lack of volatility of the traditionally employed reagents (i.e., tetraalkylammonium salts or alkylsulfonate salts), and therefore incompatibility with the electrospray ionization mass spectrometric (ESIMS) detector as well as the frequently reported adverse effects on the ESIMS signal.[7−9] To overcome these limitations, mixed-mode chromatographic columns with C18 stationary phases embedded with sulfonic or quaternary ammonium groups have been introduced as an alternative approach[10−13] and are nowadays widely commercially available. Hydrophilic interaction liquid chromatography (HILIC) is also gaining increasing popularity for the retention of polar compounds[14,15] and is generally regarded as a better alternative to normal-phase chromatography, providing chromatographic selectivity mainly based on polar rather than hydrophobic interactions. In contrast with mixed-mode reversed-phase chromatography, ion-interaction chromatography on hydrophobic C18 chromatographic columns clearly provides more versatility and flexibility in controlling the retention of charged analytes as well as the selectivity of separation and might be in some scenarios the most desirable option for tackling a specific chromatographic problem. Consequently, there is still a driving force for the development and employment of novel reagents that can provide variable selectivity and overcome the disadvantages notoriously associated with this technique. A

Received: May 18, 2020
Accepted: June 22, 2020
Published: June 22, 2020



https://dx.doi.org/10.1021/acs.analchem.0c02138
Anal. Chem. 2020, 92, 10121–10128

typical example of the exploitation of the properties of fluorinated compounds in ion-interaction chromatography involves the use of the fluorinated carboxylic acids, including the widely used trifluoroacetic acid, with the main advantages of these anionic ion-interaction reagents over their sulfonic acid counterparts being their volatility and therefore compatibility with ESIMS detection as well as their relatively short equilibration times.[16,17] More recently, the use of fluorinated alcohols as weakly acidic volatile anionic ion-interaction reagents has been described.[18,19] Volatile cationic di- and trialkylamine ion-interaction reagents were also described as alternatives to tetraalkylammonium salts.[20] Despite providing significantly higher sensitivity than tetraalkylammonium salts in ESIMS, these reagents were still associated with signal suppression by 3 to 4 fold,[8] and similarly to tetraalkylammonium, their chromatographic equilibration times are proportional to their carbon content and therefore ion-interaction capacity, with the most common compromise in this respect being triethylamine.[21]

Driven by some of the positive aspects and widespread use of the fluorinated carboxylic acids in reversed-phase chromatography, the aim of the present work was to investigate the use of commercially available fluoroalkylamine compounds as cationic ion-interaction reagents. This application of the fluoroalkylamines was not described before, and there has been a single report about the incorporation of a fluoroalkylamine in liquid chromatography, in which Kamiusuki et al. briefly mentioned the addition of heptafluorobutylamine to the mobile phase as a "masking agent of the adsorption point" within the course of a study that involved investigating the retention behavior of organic compounds on a polyfluoroalkylsilane stationary phase in an attempt to achieve an improvement in the separation of three fluoroaniline isomers.[22]

EXPERIMENTAL SECTION

Example Analytes. A group of 12 compounds were selected as model analytes, including compounds that are permanently charged or ionizable and therefore poorly retained on a C18 reversed-phase chromatographic column and would benefit from ionic interactions. Among the chosen compounds were analytes of particular interest such as herbicides fosamine, glyphosate, and ethephon as well as common arsenic compounds dimethylarsinic acid, methylarsonic acid, and arsenic acid. Furthermore, the neutral compound thiourea and the glyphosate degradation product aminomethylphosphonic acid, which is zwitterionic at pH < 4.5 (pK_a = 0.9, 5.6, and 10.2), were included. Chemical structures and pK_a values for the group of test analytes included in the present work are shown in Figure 1. The compounds were purchased from Sigma-Aldrich (Steinheim, Germany) and prepared in pure water (18.2 MΩ cm) at a concentration in the range of 0.1−15 mg L^{-1} in a mixture which was used for the chromatographic investigations (see below).

Instrumental Conditions. For the chromatographic separation, an Agilent 1100 chromatographic system (Agilent Technologies, Waldbronn, Germany) consisting of a quaternary pump (G1311A), an autosampler (ALS G1367C), a degasser (G1379A), a COLCOM (G1316A) column compartment, and an ALSTherm (G1330B) sample cooler were employed.

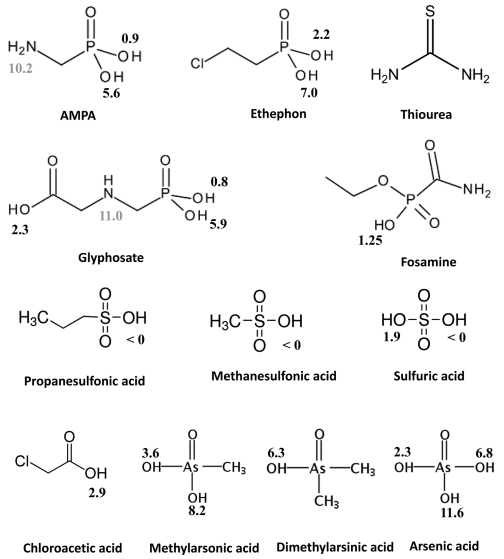


Figure 1. Chemical structures and p*K*a values of the test analytes included in the present study. Note that thiourea and dimethylarsinic acid are neutral and aminomethylphosphonic acid is zwitterionic at pH 4.0.

For chromatographic detection, an element-selective detector consisting of an inductively coupled plasma tandem mass spectrometer (ICPMS/MS, Agilent 8900 ICPQQQMS, Agilent Technologies, Waldbronn, Germany) was employed to detect the phosphorus- and sulfur-containing compounds. Coupling the ICPMS/MS with the chromatographic column was performed using PEEK capillary tubing (0.127 mm i.d. and ca. 30 cm in length) connecting the outlet of the chromatographic column with the AriMist PEEK nebulizer. Further components of the ICPMS/MS system included a glass Scott double-pass spray chamber, a Ni/Cu sampler and skimmer cones, and a quartz plasma torch with an inner diameter of 2.5 mm. The ICPMS/MS was operated in reaction cell mode with 0.3 mL min^{-1} oxygen as the reaction gas, and mass transitions 32 → 48, 31 → 47, and 75 → 91 were monitored for the detection of the sulfur-, phosphorus-, and arsenic-containing compounds, respectively.

To investigate the influence of the fluoroalkylamines on ESIMS detection, the ESIMS signal for the tested ionizable organic compounds was monitored on an Agilent 6120 single quadrupole ESIMS system coupled with a 1260 HPLC binary system (Agilent Technologies, Waldbronn, Germany). The selected compounds were fosamine, glyphosate, ethephon, chloroacetic acid, methanesulfonic acid, and propanesulfonic acid, which are easily detectable in negative ion mode. Each compound was monitored after flow injection at four different injection amounts spanning 1 order of magnitude. The injected amounts for the different compounds at the different levels were within the overall range of 0.1−10 ng, which was sufficient to yield S/N > 50 for the different tested compounds under unified default ESIMS settings: drying gas flow, 12.0 L/min; nebulizer pressure, 2.4 × 10^5 Pa; drying gas temperature, 350 °C; and capillary voltage, −3000 V. The single ion-monitoring mode was used to monitor the following m/z values: 93 (chloroacetic acid), 95 (methanesulfonic acid), 123 (propanesulfonic acid), 143 (ethephon), and 152 (fosamine), and 168 (glyphosate). Each injection was performed in triplicate. The linearity was checked for each compound under each carrier solution tested with correlation coefficients

https://dx.doi.org/10.1021/acs.analchem.0c02138
Anal. Chem. 2020, 92, 10121−10128

in the range of 0.9970−0.9999. For flow injection analysis, the sensitivities (slopes) for different dilutions of various carrier solutions were compared, namely, 15 mM ammonia, triethylamine (TEA), trifluoroethylamine (TFEAm), pentafluoropropylamine (PFPAm), heptafluorobutylamine (HFBAm), and nonafluoropentylamine (NFPAm), all adjusted with roughly equal amounts (±10%) of formic acid (ca. 23 mM) to a buffered pH of 4.00 (±0.05). Multiple dilutions of the buffered carrier solutions were produced by online mixing with pure water (18.2 MΩ cm) using the HPLC system yielding tested fluoroalkylamine concentrations of 1.3, 2.6, 6.5, and 13 mM. Ammonia (25% w/w), TEA (>99.5%), and TFEAm (>99.5%) were purchased from Sigma-Aldrich (Steinheim, Germany). PFPAm (>97%) and HFBAm (>95%) were purchased from TCI (Tokyo, Japan). NFPAm (>97%) was purchased from Manchester Organics (Manchester, U.K.).

Chromatographic Conditions. All chromatographic separations were performed on a reversed-phase C18 column (YMC Triart-C18, 3.0 mm i.d. × 150 mm long, 3 μm particle size, 1.0−12 pH stability range, compatible with 100% aqueous mobile phases). The ion-interaction capacity of different fluoroalkylamines belonging to a homologous series of fluorinated linear alkyl chains was investigated in comparison with ammonia and TEA under identical conditions (10 mM concentration and pH 4.0, adjusted with roughly equal amounts of formic acid). The chemical structures and key properties for the investigated fluoroalkylamines are shown in Figure 2. On the basis of initial chromatographic results, HFBAm was found to be a favorable choice because it provided a very good compromise between the equilibration time and retention capacity. Therefore, in those few instances where the members of the homologous series were expected to display comparable patterns, further experiments were carried out using this eluent as a representative. These included testing the concentration dependency of the retention behavior of HFBAm within the range of 0.5−20 mM (adjusted to pH 4.0 with formic acid), the effects of the pH-dependent ionization of HFBAm (pK_a ca. 5.7[23]) on its retention behavior (pH 4.5, 5.5, and 6.5, adjusted with formic acid), and the effects of the concentration of added formate (0−60 mM, adjusted to pH 4.0 with ammonia) on the retention capacity of HFBAm (pH 4.0, adjusted with formic acid). General chromatographic conditions used throughout the study were the following, unless otherwise stated: injection volume, 2.0 μL; column temperature, 40 °C; and mobile phase flow rate, 0.8 mL min^{-1}. The detailed chromatographic conditions can be found for each experiment in the respective figure caption. All chromatographic separations and mass spectrometric investigations were performed using 100% aqueous mobile phases/carrier solutions.

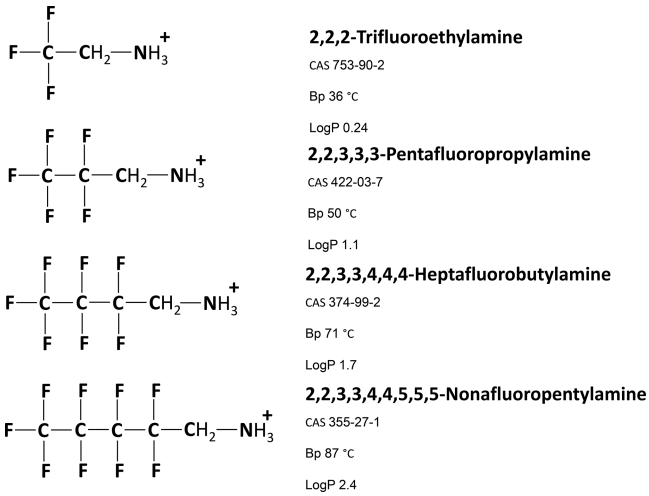


Figure 2. Chemical structures of a homologous series of linear fluoroalkylamines investigated in the present study. The Chemical Abstracts Service (CAS) registry number, the boiling point (bp) as an indication of volatility, and the log *P* value as a measure of hydrophobicity are shown. The amino groups on these compounds have a pK_a of 5.5−6.0 and are therefore >90% protonated at pH < 4.5.

RESULTS AND DISCUSSION

The employment of a fluoroalkylamine as an ion-interaction reagent was not investigated before. Our choice of fluoroalkylamines to be included in the present study was based on three interrelated criteria: (1) water solubility/miscibility at concentrations normally employed in ion-interaction chromatography (1−10 mM) under acidic pH values where the fluoroalkylamine is positively charged; (2) volatility and a sufficiently low boiling point (<100 °C) to achieve the advantage of compatibility with chromatographic detectors requiring volatile eluents; and (3) a carbon chain length of <6 to minimize the column equilibration time. Among possible fluoroalkylamines that can fulfill these criteria, a homologous series of linear fluorinated carbon chains were commercially available, including trifluoroethylamine (TFEAm), pentafluoropropylamine (PFPAm), heptafluorobutylamine (HFBAm), and nonafluoropentylamine (NFPAm), and these reagents were therefore selected. (See Figure 2 for chemical structures and key properties.) These reagents were tested and compared in terms of chromatographic behavior with ammonia and with triethylamine, a commonly employed cationic ion-interaction reagent, under identical experimental conditions.

As model analytes, the chromatographic separation of which would benefit from ion-interaction reagents, a range of compounds including those with practical significance and major interest such as phosphorus herbicides and arsenic metabolites were selected. The compounds were selected to possess various properties and charge states: (1) strongly ionizable negatively charged compounds (arsenate, sulfate, propanesulfonic acid, and methanesulfonic acid), (2) zwitterionic under the appropriate pH values (aminomethylphosphonic acid and glyphosate), (3) neutral under practical pH values (thiourea), and (4) weakly ionizable negatively charged (or conditionally neutral) under the appropriate pH values (fosamine, ethephone, chloroacetic acid, dimethylarsinic acid, and methylarsonic acid). The chemical structures and the pK_a values governing the ionizability of these compounds are shown in Figure 1.

The fluoroalkylamines showed a significant enhancement of the retention of the test analytes harboring a net negative charge at the tested pH 4.0 relative to ammonia under identical conditions, with HFBAm and NFPAm clearly showing superior retention over triethylamine as well, whereas the retention of the practically neutral (thiourea), zwitterionic (aminomethylphosphonic acid), and conditionally neutral (dimethylarsinic acid) compounds remained unchanged (Figure 3 and Supporting Information Figure S1), which is consistent with the expected behavior from an ion-interaction reagent involving the interaction between the negatively

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Anal. Chem. 2020, 92, 10121−10128

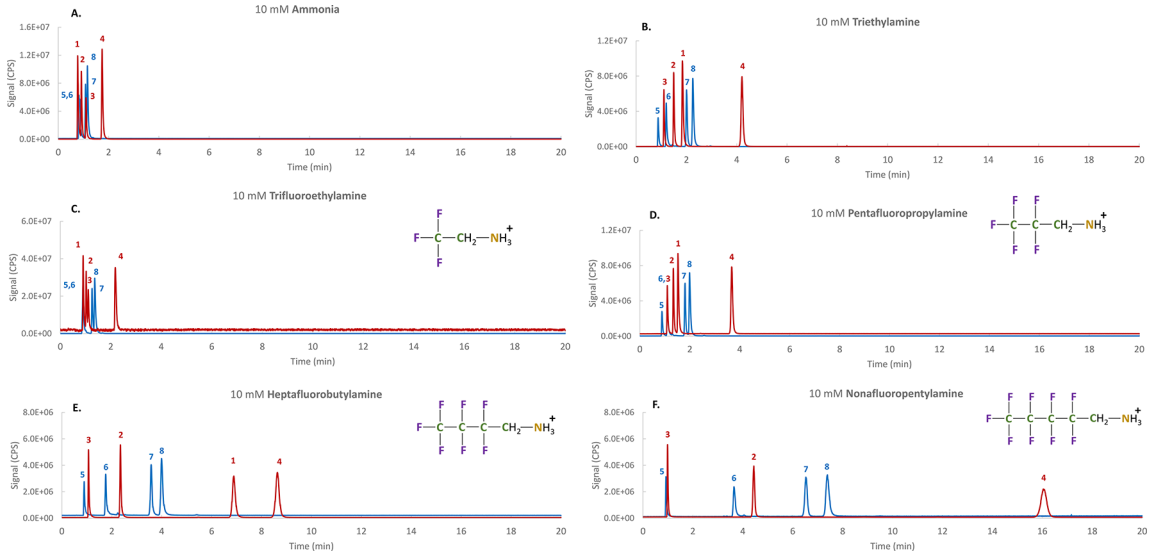

Figure 3. Comparison of ammonia (A), triethylamine (B), and various fluorinated amines (C−F) in terms of the ion-pairing capacity on a reversed-phase C18 column. A separation test mixture containing neutral/zwitterionic or negatively charged phosphorus- and sulfur-containing compounds at concentrations in the range of 5.0−15 mg S/P L^{-1} was employed. Additional arsenic compounds were also tested (Supporting Information Figure S1). The red trace indicates the sulfur compounds, namely, sulfate (1), methanesulfonic acid (2), thiourea (3), and propanesulfonic acid (4). The blue trace shows the phosphorus compounds: aminomethylphosphonic acid (5), glyphosate (6), fosamine (7), and ethephon (8). The chromatographic conditions were as follows: stationary phase, YMC Triart-C18 (3.0 i.d. × 150 mm long, 3 μm particle size, stable in pH 1.0−12 range, compatible with 100% aqueous mobile phases); column temperature, 40 °C; mobile phase flow rate, 0.8 mL min^{-1}; injection volume, 2.0 μL; mobile phase, several eluents were tested and compared, namely, ammonia, triethylamine, trifluoroethylamine, pentafluoropropylamine, heptafluorobutylamine, and nonafluoropentylamine. All contained the eluent (10 mM) under investigation with pH adjusted to 4.0 (±0.05) with roughly equal concentrations of formic acid (ca. 15 mM). The void time was ca. 0.7 min. Note the change in selectivity with increasing hydrophobicity of the ion-pairing reagent, especially between propanesulfonic acid (4) and sulfate (1). Sulfate (1) was observed as a broad peak at RT for ca. 45 min under nonafluoropentylamine.

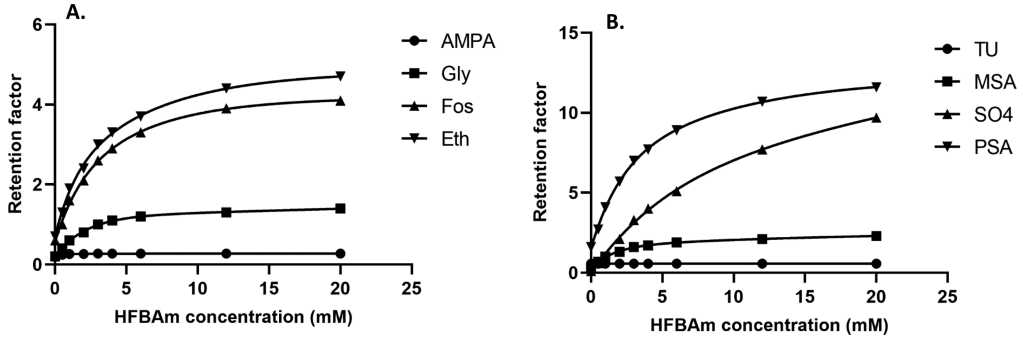

Figure 4. Concentration-dependent retention behavior of heptafluorobutylamine (HFBAm). The graphs show the relationship between increasing concentrations of HFBAm and the retention factor of various phosphorus (A) and sulfur (B) compounds, namely, sulfate (SO_4), methanesulfonic acid (MSA), thiourea (TU), propanesulfonic acid (PSA), aminomethylphosphonic acid (AMPA), glyphosate (Gly), fosamine (Fos), and ethephon (Eth). The chromatographic conditions were as follows: stationary phase, YMC Triart-C18 (3.0 i.d. × 150 mm long, 3 μm particle size, stable over the pH range of 1.0−12, compatible with 100% aqueous mobile phases); column temperature, 40 °C; mobile phase flow rate, 0.8 mL min^{-1}; injection volume, 2.0 μL; mobile phase, to maintain a sufficient buffering capacity across the different experiments, all mobile phases tested contained 5 mM ammonium formate buffer adjusted to pH 4.0 with formic acid, with the addition of various amounts of HFBAm by online mixing. The pH of added HFBAm was preadjusted with formic acid to pH 4.0. Note the more rapid increase in the retention of the inorganic doubly charged sulfate. The retention factor of the neutral reference compounds (AMPA and thiourea) remained unchanged.

charged groups on the tested compounds and the protonated positively charged amino groups on the fluoroalkylamines. Furthermore, the retention enhancement on the hydrophobic C18 stationary phase was proportional to the hydrophobicity of the fluoroalkylamine and followed the order TFEAm (log P = 0.24) < PFPAm (log P = 1.1) < HFBAm (log P = 1.7) < NFPAm (log P = 2.4) (Supporting Information Figure S2), which is in agreement with the general trends for ion-interaction reagents.[24−26]

The investigated fluoroalkylamines are members of a homologous series and share some key properties, particularly ionizability (pK_a 5.5−6.0), and would therefore be expected to show similarities or predictable differences in some respects. In these few instances, the choice was to focus on HFBAm since this reagent showed superior retention behavior over TFEAm and PFPAm (Figure 3) plausibly due to its higher hydrophobicity. Although NFBAm exhibited stronger retention behavior, the HFBAm, not surprisingly, was observed to display faster column equilibration times due to the lower

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Anal. Chem. 2020, 92, 10121−10128

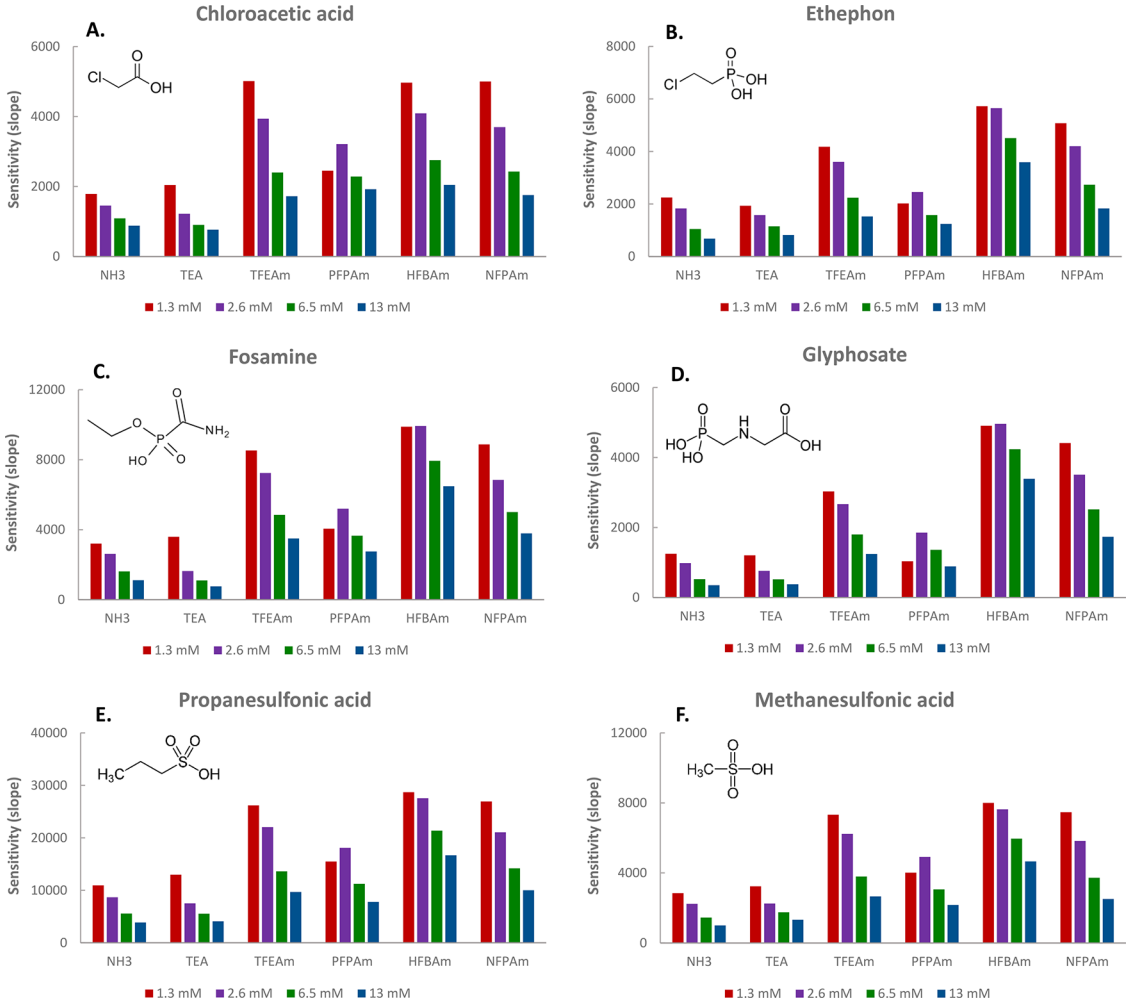

Figure 5. Effects of the fluorinated amines on the sensitivity for ESIMS detection for a few of the tested compounds (A–F, see labels). The signal for the test compounds was monitored after flow injection using various dilutions of a carrier solution containing 15 mM ammonia (NH_3), triethylamine (TEA), trifluoroethylamine (TFEAm), pentafluoropropylamine (PFPAm), heptafluorobutylamine (HFBAm), or nonafluoropentylamine (NFPAm), all adjusted to a buffered pH 4.00 (±0.05) with ca. 23 mM formic acid. The flow injection was carried out at a flow rate of 0.2 mL min^{-1} using a 5 m stainless steel restrictive capillary. Multiple replicates (n = 3) of varying injection amounts for the various test compounds (varied within the overall range of 0.1–10 ng depending on the compound to ensure a S/N ratio >50 under unified ESIMS settings) were recorded, and the average signal (as the peak area) was used to obtain the slope values (shown on the Y axis). The signal RSD% of multiple replicates was typically within the range of 2.0–7.0%. The correlation coefficients (R^2) of the calibration graphs which spanned a 10-fold difference in the injection amount were within the range of 0.997–0.999. Default instrumental ESIMS settings were used for all experiments: drying gas flow, 12.0 L/min; nebulizer pressure, 2.4 × 10^5 Pa; drying gas temperature, 350 °C; and capillary voltage, −3000 V. The single ion-monitoring mode was used to monitor the following m/z values: 93 (chloroacetic acid), 95 (methanesulfonic acid), 123 (propanesulfonic acid), 143 (ethephon), and 152 (fosamine).

hydrophobicity (ca. 20 column volumes vs 47 column volumes at pH 4.0 and 10 mM concentration), which was one of the key targets for investigating the potential of the fluoroalkylamines. The relationship between the retention factor of the tested negatively charged compounds and the HFBAm concentration followed an exponential profile, in line with a type I Langmuir adsorption isotherm (Figure 4). The graphs in Figure 4 also show that the optimal concentration range of HFBAm for maximum and relatively concentration-independent retention behavior is 10–20 mM (at pH 4.0, which provides virtually complete ionization of amino group on the fluoroalkylamines).

The presence of the strongly electron-withdrawing fluorine atoms on the β sites of the fluoroalkylamines significantly lowers their basicity and pK_a values to 5.5–6.0.[23] The ion-interaction capacity of the investigated fluoroalkylamines is therefore dependent on their ionization state and is maximized at a mobile phase with pH < 5.0 (Supporting Information Figure S3), which is suitable for formate/acetate buffering systems. The removal of the fluorine atoms at the β sites and the associated increase in the pK_a would extend the operating pH range for the ion-interaction capacity of the investigated fluoroalkylamines. Such derivatives were, however, not tested in the present work because they were not commercially available. However, the low basicity of the investigated fluoroalkylamines might in fact be advantageous in some cases, since varying the pH and thereby the ionization on the fluoroalkylamines may provide alternative selectivity due to the various possible degrees of protonation of the amino groups on the adsorbed fluoroalkylamines and the associated various contributions of other interaction mechanisms such as hydrogen bonding. Together with the C–F bonds, the exposed

https://dx.doi.org/10.1021/acs.analchem.0c02138
Anal. Chem. 2020, 92, 10121–10128

amino groups and their variable degree of ionization, which is easily controllable within the wide buffering range of a fluoroalkylamine−formate system (3.0−7.0), may render the retention mechanism with the described fluoroalkylamines rather complex but may also confer novel and controllable selectivity relative to the traditionally employed alkylamine reagents. It should be emphasized, however, that attention has to be paid to the water solubility/miscibility of highly hydrophobic, weakly basic fluoroalkylamines. The most hydrophobic fluoroalkylamine in the tested series, NFPAm, showed water miscibility at concentrations of at least 100 mM at acidic pH values that permit virtually complete ionization (i.e., pH < 4.5), but the miscibility of NFPAm quickly decreased in pure water (18.2 Ω cm, pH ca. 6.5) to a roughly estimated 10 mM. For key parameters, including toxicity, chemical safety, and the experimentally determined miscibility in pure water, see Supporting Information Table S1.

Ion-interaction/ion-pair chromatography is known to be associated with several drawbacks. First, due to the adsorption process of the ion-interaction reagent on the hydrophobic stationary phase, the column equilibration times are notoriously long and proportional to the hydrophobicity of the ion-interaction reagent and in turn the ion-interaction capacity. Therefore, a compromise between the ion-interaction capacity and equilibration time has to be made. In our experience, ion-interaction reagents with carbon atom counts of more than six generally require equilibration with 50−100 column volumes to yield stable retention times. It is also commonly recommended to dedicate a certain reversed-phase column to ion-interaction chromatography due to not only the long equilibration times but also the difficulty of completely removing highly hydrophobic ion-interaction reagents (e.g., the traditionally employed tetrabutylammonium salts) from the stationary phase, which has the consequence of persistently modifying the retention properties of the column as well as generating a permanently high background at the m/z of the ion-interaction reagents when ESIMS is employed as the detector due to their slow bleeding from the column. The fluoroalkylamines displayed promising behavior in terms of their equilibration and washout times. HFBAm showed a good compromise between the ion-interaction capacity and equilibration time with 15−35 column volumes (depending on the pH and HFBAm concentration) being sufficient to yield stable retention times (RSD < 0.5%). The repeatability in retention time for all of the fluoroalkylamines tested was within the range of 0.1−1.5% (expressed as the RSD % (n = 3)). Supporting Information Table S2 shows retention time repeatability data for all of the fluoroalkylamines tested at 10 mM concentration and pH 4.0, following complete column equilibration achieved in approximately 10, 20, and 50 column volumes for TFEAm/PFPAm, HFBAm, and NFPAm, respectively (Supporting Information Table S2). The retention times on the C18 column prior to first exposure to the fluoroalkylamines (with 5 mM ammonium formate (pH 4.0) as the mobile phase) were compared with those after equilibration with 10 mM HFBAm (pH 4.0), followed by switching back to the HFBAm-free mobile phase (Supporting Information Figure S4). The recovery process was dynamically monitored by consecutive injections in small time intervals. The column showed relatively fast recovery to its original retention properties in ca. 30 min (40 column volumes), without resorting to a washout with an organic mobile phase (Supporting Information Figure S4).

Another general disadvantage of ion-interaction reagents is their ESIMS behavior. The volatility of di- and trialkylamines renders them compatible with ESIMS detection,[20,27] and while these reagents provide significantly higher ESIMS sensitivity than the nonvolatile tetraalkylammonium salts, which is associated with severe signal suppression (>20 fold),[8] they were still reported to consistently suppress the signal (e.g., of sulfonated compounds) by 3- to 4-fold.[8] The influence of all of the tested fluoroalkylamines on the ESIMS sensitivity for the detection of several ionizable compounds was investigated. Remarkably, the fluoroalkylamines showed a consistent enhancement of sensitivity by 3−5-fold for all of the tested negatively charged compounds, relative to ammonia when compared under identical conditions (Figure 5). It is noteworthy that the general trend of decreasing sensitivity upon moving to higher eluent concentration is likely to be mainly due to the accompanying increased concentration of the formate counterion, which may compete with the negatively charged tested compounds as this decrease was clearly observed for ammonia (Figure 5). On the other hand, excessively low eluent concentration results in low conductivity of the carrier/mobile phase and may negatively affect sensitivity. This may explain why PFPAm showed a sensitivity peak pattern (i.e., the sensitivity was found to be highest at 2.6 mM and lower at lower or higher concentration (Figure 5)). The optimal concentration for maximum sensitivity also appears to be different for each fluoroalkylamine depending on the enhancement capability (e.g., around 1.3 mM for HFBAm) (Figure 5B−F). The mechanism of the observed enhancement is not clear but is likely related to two distinguishing factors for these ion-interaction reagents: the very low boiling points (TFEAm, 36 °C; PFEAm, 50 °C; HFBAm, 71 °C; and NFPAm, 87 °C) and their relatively low basicity/ionizability (pK_a 5.5−6.0). Our tentative explanation is that due to the high volatility the possibly formed ion pairs would have enhanced transfer to the gas phase, relative to the ionized native analyte. The low basicity (and ionization) of the fluoroalkylamines may facilitate the dissociation of the ion pair in the gas phase, resulting in an overall increase in the sensitivity. It is worth mentioning that the fluorinated carboxylic acids which are relatively less volatile (e.g., TFA, 72 °C; PFPA, 97 °C) but much more ionizable (pK_a < 1.0) are known to be associated with significant ESIMS signal suppression of basic analytes.[7] Future work will involve a more detailed investigation of the observed enhancement and its possible dependency on ionization source parameters and ionization source geometry.

Strongly ionizable alkylamines are also notoriously known to be associated with persistent contamination of the ESIMS system and memory effects.[28] During a washout procedure, a persistent signal at m/z 102 (TEA) for at least 6 h (>200 000 counts) was observed, whereas the signal at m/z 200 (HFBAm) quickly dropped to <1000 under identical conditions (carrier, 70% methanol in pure water; pH ca. 6.0). The more rapid decrease in the signal for HFBAm relative to TEA indicates faster and virtually complete washout in <40 min (Supporting Information Figure S5).

Two discrete mechanisms of retention in ion-pair/ion-interaction chromatography were proposed.[26,29−31] One involves the interaction of the reagent with the analyte in the mobile phase followed by adsorption of the formed hydrophobic ion pair on the stationary phase,[24,32] while the other involves adsorption of the ion-pairing reagent on the stationary

https://dx.doi.org/10.1021/acs.analchem.0c02138
Anal. Chem. 2020, 92, 10121−10128

phase and a dynamic ion-exchange process.[33] It is generally accepted that the overall retention in ion-pair chromatography involves elements of both mechanisms, the relative contribution of which depends on the analyte, the interacting reagent, and the chromatographic conditions.[5,32,34] Low et al. reported that an increase in the ionic strength of the mobile phase shifts the dominance toward the ion-pair formation mechanism over the dynamic ion-exchange mechanism.[35] The effects of increasing concentrations of added ammonium formate (0−60 mM, pH 4.0) on the retention under 10 mM HFBAm-formate (pH 4.0) were investigated, and a clear decrease in the retention with increasing formate concentration was observed (Supporting Information Figure S6), with the retention of the hydrophilic doubly charged sulfate being the most sharply suppressed by added formate (Supporting Information Figure S7), which can be interpreted as an indication of a strong contribution of a dynamic ion-exchange mechanism.

It is evident that the historical and more commonly used term "ion-pairing reagent" misrepresents the large contribution of the dynamic ion-exchange mechanism, which might be particularly relevant in the case of the fluoroalkylamines, given that the present data indicates that these reagents show strong dynamic ion-exchange behavior. Alternative, more general terms such as ion-interaction reagents were justifiably coined to address this issue,[5] which was therefore adapted in this article.

CONCLUSIONS

The presented group of the fluoroalkylamines forms a new class of ion-interaction reagents and was shown to address several of the common disadvantages associated with ion-pair chromatography, featuring an ESI-MS signal enhancement, high volatility, and relatively fast equilibration and washout times. These advantages have the potential to change the current general attitude toward ion-interaction chromatography. The extension of the present work to other fluorinated amines of different general structures compared to those investigated in the present work may be warranted. Application-oriented studies involving the described fluoroalkylamines will undoubtedly follow.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c02138.

The separation of additional arsenic compounds; retention vs carbon chain length of the fluoroalkylamine; pH dependency of the retention under heptafluorobutylamine; retention recovery following washout; ESI-MS washout time; chromatograms showing the effects of added formate concentration; log[formate] vs log k; general characteristics of the fluoroalkylamines; and retention time repeatability (PDF)

AUTHOR INFORMATION

Corresponding Author

Bassam Lajin − *Institute of Chemistry, Analytical Chemistry for Health and Environment, University of Graz, 8010 Graz, Austria*; orcid.org/0000-0002-7501-0014; Email: Bassam.lajin@uni-graz.at

Author

Walter Goessler − *Institute of Chemistry, Analytical Chemistry for Health and Environment, University of Graz, 8010 Graz, Austria*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.0c02138

Notes

The authors declare no competing financial interest.

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https://dx.doi.org/10.1021/acs.analchem.0c02138
Anal. Chem. 2020, 92, 10121−10128